Final Report

ENVIRONMENTAL CONTAMINANTS PROGRAM
ON-REFUGE INVESTIGATIONS SUB-ACTIVITY

Starlings as Avian Model and Monitors of Remedial Actions on Crab Orchard National Wildlife Refuge

Project ID: 3C16
(filename: Final Report2000a.wpd)

By

Richard S. Halbrook
Christine Arenal
Shelley Grey
Cooperative Wildlife Research Laboratory
Southern Illinois University
Carbondale, IL 62901

For

United States Fish and Wildlife Service
Crab Orchard National Wildlife Refuge
Marion, IL

December 2000

EXECUTIVE SUMMARY

We used the starling to monitor accumulation and affects of PCBs in avian species at the PCB Operable Unit on Crab Orchard National Wildlife Refuge (CONWR) prior to and after remediation. This report addresses results of the post remediation study, in addition, comparisons with pre-remediation results are included. Prior to remediation, mean Aroclor 1254 concentration in carcasses of starling chicks collected from the Area 9 Building Complex (AB) and Area 9 Landfill (AL) sites were 5.9 and 52.5 mg/kg, respectively (Arenal 1997). The mean Aroclor 1254 concentrations in chick carcasses were 2.6 and 2.5 mg/kg, respectively, at each of these sites in 1999 (post remediation). Because PCB congeners differ in their biodegradation and bioaccumulation patterns and are of different toxicities to wildlife, toxic equivalent factors (TEQs) were used to evaluate potential toxicity (Safe 1990). The TEQs of the PCBs present in starling chick carcasses indicated an order of magnitude decrease in toxicity at the PCB sites after remediation. These results indicate that remedial action successfully reduced PCB concentrations in starling chicks collected from the AB and AL sites by approximately 66 and 95%, respectively. However, the mean Aroclor 1254 concentration measured in chick carcasses collected from remediated sites in 1999 continued to be significantly greater than concentrations measured in carcasses of chicks collected from a reference site (0.5 mg/kg) during that year.

Stickel et al. (1984) reported a mean PCB concentration of 179 ppm in adult starling carcasses at the point where 50% of starlings exposed to PCBs in the diet died. Although similar data are not available for starling chicks, there is no indication from our studies that PCB concentrations measured in chicks collected from CONWR were associated with excessive mortality (i.e., mortality in 50% of chicks). Although the mean Aroclor 1254 concentration measured in chicks at the AL site prior to remediation (52.5 mg/kg) was of concern, the low number of productive nests at this site ($n = 1$) precluded a reliable assessment of percent mortality at this carcass concentration. However, observed differences in adult provisioning rate (number of times adults came to the nest per hour) and chick mortality between PCB and reference sites prior to remediation suggests that PCB concentrations at that time were sufficient to result in adverse effects. There were no reproductive, behavioral, or mortality differences observed after remediation that would suggest the continuation of adverse effects.

The use of the starling as a model for evaluating potential effects of contaminants on avian species has been successful and demonstrates the value of biological monitoring as a mechanism for evaluating environmental contamination and the efficacy of remediation. The lack of biologically significant differences in adult behavior and fecundity among starlings nesting at remediated and reference sites suggest that potential adverse effects to avian species are no longer of concern.

INTRODUCTION

Polychlorinated biphenyl (PCB) and heavy metal contamination of the eastern section of Crab Orchard National Wildlife Refuge (CONWR), Illinois, was detected during the late 1970's and early 1980's (O'Brien and Gere 1988). In 1984, the United States Environmental Protection Agency (USEPA) recommended 7 sites within CONWR for inclusion on the Superfund National Priority List for toxic waste cleanup. Previous studies at CONWR reported accumulation and potential effects of PCBs on European starlings (*Sturnus vulgaris*) nesting at one of the PCB contaminated site on the refuge (McKee 1993). Studies by Arenal and Halbrook (1997) also reported greater PCB concentrations in starling chicks collected from contaminated sites on CONWR compared to reference sites. They observed significantly higher chick mortality, a reduction in nest attentiveness (chick provisioning rate), and reduced fledging success in starlings nesting at PCB sites compared to those nesting at reference sites. Following these earlier studies, remediation of PCB sites has occurred and this report addresses results from further studies using the starling as a biomonitor of the effectiveness of that remediation.

Starlings as Monitors

There are a number of advantages to using avian species as monitors (Furness 1993). Avian species are relatively easy to identify, their classification and systematics are well established, and large amounts of data have previously been collected regarding avian populations. Because avian species accumulate contaminants from their food and water, and through incidental ingestion of soil and sediment, they integrate toxic compounds from multiple sources (Selikoff 1972, Feare 1984). In addition, Fox (1993) reported that congenital malformations were uncommon in most wild bird populations and the occurrence of birth defects may serve as a sensitive biomarker for the presence of toxins in the food chain.

The European starling was introduced in North America in 1880 and within 80 years of its introduction, it became one of the most numerous birds in North America, with a breeding area extending from arctic Canada to the sub-tropics of Mexico (Kessel 1957). Starlings have characteristics suitable for environmental monitoring, including a tolerance for human activity, feeding on invertebrates in the top 5 cm of soil, and utilization of nest boxes (Kessel 1957, Feare

1984, Cabe 1993).

STUDY SITES

Crab Orchard National Wildlife Refuge is located between Marion and Carbondale, Illinois, and covers approximately 17,600 hectares of forest, wetlands, and grasslands (USEPA 1988). This area was extensively farmed in the 1920s and 1930s, however the soil was infertile and badly eroded causing the farming industry to fail. In 1936, the Resettlement Administration acquired these depleted lands with the intent of constructing 3 lakes for an industrial water supply and recreation. The onset of World War II shifted ownership of this project to the War Department who established the Illinois Ordnance Plant, a munitions production facility. At the end of World War II, the War Department transferred this land to the Department of the Interior for use as a national wildlife refuge (O'Brien and Gere 1988). Crab Orchard National Wildlife Refuge was established on August 5, 1947, with 4 management objectives: wildlife management, recreational use, agricultural development, and industrial operations (USEPA 1988). After the war, various companies moved onto CONWR to occupy buildings previously used for manufacture of wartime materials. These companies have been involved in manufacturing munitions, metal fabrications and plating, and the manufacture of printing inks and electrical components. Among these industries was the Sangamo Electric Company, Capacitor Division. The disposal of wastes from electrical capacitor manufacturing by Sangamo Electric Company resulted in PCB contamination of several sites on CONWR (O'Brien and Gere 1988). During the 1980's, investigative studies identified PCB contamination at the Area 9 Landfill (AL) and Area 9 Building Complex (AB), and remediation of these sites began in 1996. The current study measured the accumulation and effects of PCBs in starlings collected from these remediated sites relative to starlings collected from reference sites.

Area 9 Landfill

From 1946-1962, the AL was a manufacturing site leased to the Sangamo Electric Company and is currently leased by Primex Corporation, a munitions manufacturer. This approximately 1 ha landfill is located 100 m south of Crab Orchard Lake, and was used by

Sangamo Electric Company for disposal of wastes from capacitor manufacturing operations. Prior to remediation, surface soil PCB concentrations in the landfill averaged 3,200 mg/kg, wet wt (O'Brien and Gere 1988).

Area 9 Building Complex

The AB site is located approximately 80 m west of the AL site and consists of 5.3 ha of land. This site was used by Sangamo Electrical Company from 1946-1962 for the manufacturing of transformers and electrical capacitors and is currently leased by Primex Corporation. PCB contamination at this site was greatest (up to 120,000 mg/kg, wet wt. in surface soils) near two buildings at the north end of the complex. Contaminants also were found in soil samples collected along two ditches where storm runoff or spills had carried PCBs from this site (O'Brien and Gere 1988).

Area P Reference Site

The area P reference (AP) site is located approximately 5 km west of the AL and AB sites on CONWR. It is a 1.2 ha field bordered on three sides by woods and a road to the north. The topography is similar to that of the contaminated sites and there is no known contamination (O'Brien and Gere 1988).

Annex Reference Site

The Wildlife Annex reference (AX) site is approximately 32 km west of CONWR, adjacent to the Cooperative Wildlife Research Laboratory Annex building on the campus of Southern Illinois University, Carbondale, Illinois. The 1.2 ha site consists of an open field bordered by trees to the south and a building to the north. No known contaminants have been reported at this site.

OBJECTIVES

Prior to remediation, the AL and AB sites had concentrations of PCBs that potentially posed a risk to wildlife (O'Brien and Gere 1988). Using the starling as an avian model, Arenal

and Halbrook (1997) reported increased chick mortality, reduced nest attentiveness, and reduced fledging success at these sites. After remediation, it was expected that environmental concentration of PCBs would decrease thus reducing the risk to avian species. The current study used the European starling as an avian monitor to evaluate the effectiveness of remedial action. The objectives of the study were to:

1. Evaluate behavior and productivity of adult starlings nesting on the remediated sites.
2. Evaluate physical condition of starling chicks collected from the remediated sites.
3. Compare pre- and post-remedial PCB concentrations and liver ethoxyresorufin O-deethylase (EROD) activity in starling chicks collected from the remediated sites.

METHODS

Nesting Behavior and Productivity

Sixty-eight starling nest boxes were placed at remediated [AL (n = 12) and AB (n = 12)] and reference sites [AP (n = 20) and AX (n = 24)]. Monitoring of nest boxes began in April, prior to the nesting season, and continued until July, the end of the nesting season. All boxes were checked at 2- 3-day intervals from nest initiation through chick hatching and daily from hatching to chick fledging. Productivity was measured by recording the number of nests constructed, number of eggs laid per nest, number of eggs hatched per nest, and number of chicks that survived 15 days post-hatch. Eggs that were abandoned or failed to hatch and chicks that died prior to 15 days of age were collected, transported to the laboratory, and archived in the event that further PCB analyses were desirable.

Following Arenal and Halbrook (1997), adult starling visitation rates at remediated and reference sites were monitored after chicks hatched. Starling nests were observed for 30 minute intervals, 3 times per week, and the number of times adults visited the nest were recorded. Visitation rates were calculated as the number of visits/hour. It was not possible to determine whether every visit by an adult to a next box involved the provisioning of chicks since food items could not always be observed; however, it was assumed that visitation rate indexed chick provisioning rate. During each nest observation period, the age of chicks and weather conditions (cloud cover and temperatures) were recorded. The time of observation at the various sites was

altered daily to avoid time-of-day bias. The period of time after chicks hatched was divided into 5 categories: 1-3 days, 4-6 days, 7-9 days, 10-12 days, and 13-15 days. The visitation rate of adults to the nest during each time period was analyzed, and nesting behavior and productivity of adult starlings were compared among remediated and reference sites.

Physical Condition

Chicks were weighed (to the nearest 0.01 g) in the field on days 3 and 9 post-hatch using a portable electronic scale. At 15-days of age, all chicks were collected and transported to the Cooperative Wildlife Research Laboratory Annex, with the exception of the AX reference site in 1999, where only 2 chicks were collected from each of 24 nest boxes . In the lab, a needle was used to prick the brachial vein, and the blood of each chick was collected in 3 heparinized hematocrit tubes. Following blood collection, hematocrit tubes were centrifuged for 15 min and the ratio of red blood cells to plasma measured. Chicks were euthanized by asphyxiation with CO_2 and 15-day-old weights were recorded, necropsies were conducted, and the gender of the bird determined. During necropsy, the liver was removed, weighed, snap frozen in liquid nitrogen, and stored at -80°C prior to analysis of EROD activity. The stomach was removed and the carcass was stored at -20°C prior to whole carcass determinations of PCB concentration (Aroclor 1254, sum of PCBs, and 20 individual congeners [CBs]). Starling chick body and liver weights, and hematocrits were compared among sites.

Chemical Analyses

PCB concentrations were determined by the Cooperative Wildlife Research Laboratory, Southern Illinois University, Carbondale, Illinois following EPA methods 3541, 3640, and 8081 with modifications for extraction, clean-up, and quantification, respectively (USEPA 1992, Arenal 1997). Carcass and egg samples were homogenized with anhydrous sodium sulfate at a ratio of 4:1, Na_2SO_4 to tissue. The mixture was blended and then extracted with hexane using a Soxtec continuous extractor. The hexane fraction was reduced using rotary evaporation and clean-up was accomplished by solvent partitioning using methylene chloride with Gel Permeation Chromatography. A Hewlett-Packard 5890 II Gas Chromatograph equipped with a

Ni-63 electron capture detector and DB5 fused silica capillary column was used for extract analysis. Quantification of Aroclor 1254 was accomplished by comparing 10 representative peaks of a standard Aroclor 1254 preparation to the same 10 peaks in extracted samples containing unknown quantities of PCBs. In addition to Aroclor 1254, 20 individual congeners were quantified, and individual congeners with concentrations above the detection limit were recorded. PCB congeners with carcass concentrations above the detection limit in at least 50% of the samples analyzed also were compared between the remediated sites during each year. For this analysis, a concentration of ½ the detection limit was assigned to those samples with detected concentrations below the detection limit. Toxic Equivalent Quotients (TEQs) also were calculated based on Toxic Equivalent Factors reported by Safe (1990) and Ahlborg et al. (1994). All results were reported on a wet weight basis.

Liver EROD activity was determined following methods of Mazel (1971) and Lubet et al. (1985). Microsomal fractions were obtained by centrifugation followed by O-dealkylation of ethoxyresorufin and measurement of resorufin formation determined using a Hitachi F-2000 Fluorescence Spectrophotometer.

Project Quality Assurance

Starling nest boxes were uniquely numbered and during collection, chicks from the same nest were placed in a carrier labeled with the corresponding nest box number, and transported to the Cooperative Wildlife Research Laboratory Annex. When individual chicks were removed from the carrier, a uniquely numbered tag was attached to it's leg.

Identification of collected chicks was recorded on chain-of-custody forms and the chain-of-custody was maintained for the entire analytical process. Data collected throughout the study were copied and archived in two locations on campus.

Samples collected for PCB and EROD analysis were analyzed with one duplicate tissue sample per batch of 10 samples. Standards (sample with a known concentration of the contaminant), spiked samples (tissue of unknown concentration to which a known concentration of the contaminant is added), and blanks (no contamination) were analyzed prior to the beginning of sample analysis and also with every batch of 10 samples. In addition, during 1998, 10 samples

were randomly selected and sent to US Fish and Wildlife, Patuxent Wildlife Research Center for PCB analysis. Correlation analysis was used to compared these results with those obtained by the Cooperative Wildlife Research Laboratory.

Statistical Analyses

Data were analyzed using SYSTAT software (SYSTAT, Inc., Evanston, Illinois). A two-way ANOVA, Tukey-Kramer multiple comparison test, or t-test was used to separate means of various parameters among or between sites as appropriate. All data were tested for normality prior to analysis using kurtosis, box plots, and by comparing the mean and median of groups of data. If data were not normally distributed, data were analyzed using an appropriate nonparametric statistical test. For data with a normal underlying distribution, a two-way ANOVA was used to evaluate differences among sites, between years, and to evaluate site by year interactions. A Tukey-Kramer multiple comparisons analysis was used to determine differences among means when significant differences in main effects were observed. The student's t-test was used to analyze individual PCB congeners between remediated sites in each year . All tests were considered significant at $P < 0.05$.

RESULTS

Behavior and Reproductive Success

The total number of nests with eggs was similar among the AL and AB remediated sites and the AX reference site during 1998 and 1999; however, there were fewer nests constructed at the AP reference site during both years (Table 1). Mean clutch size and hatching success were similar among sites ($P = 0.612$ and 0.444, respectively) and between years ($P = 0.858$ and 0.474, respectively), and the percentage of chicks surviving to 15-days post-hatch also was similar among sites ($P = 0.421$) and between years ($P = 0.938$) (Table 2).

During 1998 and 1999, there were 443 recorded visitations of adult starlings to nest boxes. Visitation rates were similar among sites ($P = 0.054$) and between years ($P = 0.690$) (Table 3). Visitation rates from 1-3 days post-hatch through 13-15 days post-hatch tended to increase across all sites during 1998 and 1999 (Figure 1).

Physical Measurements

Three- and nine-day-old chick weights were similar (P = 0.291 and 0.099, respectively) among sites, however, chick weights at both 3 and 9 days were greater (P < 0.001 and P = 0.008, respectively) during 1998 than 1999 (Table 4, Figures 2 and 3). Weights of 15-day-old starling chicks collected during 1998 and 1999 were greater (P = 0.032) in males than in females; therefore, genders were separated and compared among sites. Weights of 15-day-old male starling chicks did not differ among sites (P = 0.113), however, weights differed (P = 0.015) between years (Table 5). Fifteen-day-old male chick weights were greater in 1999 than in 1998, with the exception of those collected from the AX reference site, which did not differ between years (Figure 4). Weights of 15-day-old female starling chicks were similar between years (P = 0.137), however, weights differed among sites (P = 0.002). Female chicks collected from the AB remediated site weighed less than those collected from the AL and AX sites (Table 6, Figure 5).

One hundred and thirty-six 15-day-old starling chicks were necropsied during 1998 and 113 during 1999. During 1998 and 1999, the mean liver somatic index differed among sites (P < 0.001). The liver somatic index for chicks collected from the AB remediated site during 1998 was greater than the liver somatic index for chicks collected during that year from the AL remediated and AX reference sites, but not the AP reference site (Table 7, Figure 6). During 1999, the liver somatic index for chicks collected from the AL remediated site was less than that for chicks collected from the AX reference site. There also was a significant difference (P = 0.021) in the liver somatic index between 15-day-old chicks collected from the AX reference site during 1998 and 1999. The liver somatic index was greater in 1999 (Table 7).

PCB Data

Fifteen-day-old chick carcass PCB concentrations were determined at the AP reference site during 1998 (n = 19), however, because of low nesting activity at the AP site in 1999 (only one successful nest), PCB concentrations only were determined at the AX reference site in 1999 (n = 24). During 1998 and 1999, 38 and 25 15-day-old chicks from the AB remediated site, respectively, and 37 and 26 15-day-old chicks from the AL remediated site, respectively, were analyzed for PCBs. The sum of PCB concentrations in 15-day-old chick carcasses were similar

between the remediated sites during 1998 ($P = 0.301$) and 1999 ($P = 0.968$); however, concentrations decreased ($P = 0.001$) at both sites during that time period (Tables 8 and 9).

The sum of PCBs and individual congener concentrations in carcasses of 15-day-old starling chicks were compared between the AP reference site during 1998 and the AX reference site during 1999 (Table 10). The sum of PCB concentrations were similar (P = 0.439) between the two reference sites, and therefore, were combined for statistical comparison with remediated sites. The sum of PCB concentrations was less ($P < 0.001$) at the reference sites compared to concentrations at the remediated sites (Figure 7).

During 1998, concentrations of 8 and 9 of 20 congeners measured were detected in >50% of the samples analyzed from the AB and AL remediated sites, respectively (Table 8). There were no significant differences ($P > 0.05$) in concentrations of the 8 congeners that were detected in carcasses from both sites. During 1999, concentrations of 7 of 20 congeners measured were detected in >50% of the samples analyzed from both remediated sites (Table 9). Similar to results from 1998, there were no significant differences in concentrations of these 7 congeners between these sites.

At the AP reference site, 4 of 20 individual congeners evaluated in carcasses during 1998 were quantified in >50% of the samples analyzed, while only 1 of 20 was quantified in >50% of the samples analyzed during 1999 from the AX reference site (Table 10). The sum of TEQ values was an order of magnitude less at the AP reference site compared to remediated sites during 1998 (Tables 8 and 10). During 1999, the sum of TEQs at the AX reference site was 2 orders of magnitude less than the sum of TEQs at the remediated sites (Tables 9 and 10).

Aroclor 1254 concentrations in starling chicks were similar ($P = 0.621$) between years at all sites; however, concentrations differed ($P < 0.001$) among sites (Table 11). The AL and AB remediated sites had greater concentrations of Aroclor 1254 than the reference sites.

In addition to 15-day-old chicks, 36 chicks that died prior to 15-days of age ($n = 9$, 13, and 14 from the AX, AB, and AL sites, respectively) and 35 eggs that did not hatch ($n = 10$, 12, and 13 from the AX, AB, and AL sites, respectively) also were analyzed for PCBs. During 1998, total PCBs in carcasses of chicks that died prior to 15-days post-hatch were greater ($P < 0.001$) at remediated sites compared to the AX reference site (Table 12). However, during 1999, there was

no difference ($P = 0.082$) in total PCB concentrations in carcasses of chicks that died prior to 15-days post-hatch among remediated or reference sites; although concentrations tended to be greater in carcasses of chicks collected from the remediated sites. Sample sizes (i.e., $n = 2$ at the AX reference site), undoubtedly influenced the power of the statistical test to detect a difference among the remediated and reference sites during 1999.

Similar to results observed for chicks that died prior to 15-days of age, total PCBs in eggs that did not hatch in 1998 and 1999 differed ($P < 0.05$) among remediated and reference sites. Total PCB concentrations in 1998 and 1999 were lowest in eggs collected from the AX reference site and greatest in eggs collected from the AB remediated site (Table 13). Total PCB concentrations decreased, or remained approximately the same, in eggs collected from all sites from 1998 to 1999.

Biomarker Analysis

Liver EROD activity in 15-day-old starling chicks differed among sites within and between years (Table 14). Liver EROD activity was greater ($P = 0.002$) at the AB and AL remediated sites compared to the AP reference site during 1998. During 1999, liver EROD activity was greater ($P = 0.041$) at the AL remediated site compared to the AX reference site, however, there was no difference between EROD activity in chicks collected from the AB remediated site and the reference site. All sites had greater ($P \leq 0.001$) liver EROD activity during 1999 compared to 1998.

Hematocrit values at the AB remediated site differed ($P < 0.001$) from those at the AL remediated and AX and AP reference sites during 1998 (Table 15). During 1999, only hematocrits at the AP reference site and AL remediated site differed ($P = 0.001$). Hematocrit values also decreased ($P = 0.003$) between years at the AX reference site.

Quality Control

Ten randomly selected samples sent to a Patuxent Analytical Control Facility for PCB analysis were compared to results obtained by the Cooperative Wildlife Research Laboratory. Total PCBs and Aroclor 1254 concentrations in samples analyzed by the two facilities were

positively correlated ® = 0.974 and 0.977, respectively) (Table 16).

DISCUSSION

Aroclor 1254 concentrations, sum of PCBs, and concentrations of individual congeners quantified in carcasses of 15-day-old starling chicks collected from remediated sites were less than those previously reported by Arenal and Halbrook (1997) in 15-day-old starling chicks collected from these sites prior to remediation (Table 17). The decrease in PCB concentrations in starling chicks documents the success of the remedial process. However, concentrations of PCBs in chicks collected from remediated sites remains greater than concentrations in chicks collected from reference sites during 1998 and 1999. This is not unexpected because removal of 100% of environmental PCBs was not the remedial goal (Lombardo 1993).

Arenal (1997) reported a mean carcass Aroclor 1254 concentration of 52.5 ppm at the AL site prior to remediation. Post-remediation carcass Aroclor 1254 concentration at the AL site decreased to 2.97 ppm during 1998 and 2.49 ppm during 1999. Stickel et al. (1984) reported mean PCB concentrations of 179 ppm in adult starling carcasses at the point where 50% of the starlings died. Although similar data are not available for starling chicks, there is no indication from the current study that PCB concentrations measured in chicks collected from CONWR were associated with excessive mortality (i.e., mortality in 50% of chicks). Although the mean Aroclor 1254 concentration measured in chicks at the AL site prior to remediation (52.5 mg/kg) was of concern, the low number of productive nests at this site (n = 1) precluded reliable assessment of percent chick mortality at the carcass PCB concentrations that existed at that time.

In the current study, post-remediation concentrations of CBs 138, 153, 128, 118, 101, and 99 contributed the greatest percent of the sum of CBs at the AL and AB sites. Prior to remediation CBs 138, 153, 118, 101, 105, and 99 were most common, contributing greater than 50% of the total PCBs, at the AL and AB sites (Arenal 1997). Mora (1996) reported the predominant PCB congeners in 4 species of birds were 153, 138, 180, 110, 187, and 92 and that congeners 153, 138, and 180 were the most abundant accounting for 26 to 41% of the total PCBs. In general, the dominant congeners measured in starling chicks during our studies did not agree with those previously reported in avian species. Of particular interest, was the quantification of

the co-planar CBs 118 and 105 in starling chicks collected from study sites on CONWR.

Because PCBs differ in their biodegradation and bioaccumulation patterns and are of different toxicities to wildlife, it is difficult to accurately assess potential hazards to wildlife when interpreting tissue concentrations (Falandysz et al. 1994). Studies have indicated that non- and mono-ortho PCB congeners (co-planar congeners) are the more acutely toxic congeners, and therefore, biological samples with greater concentrations of co-planar congeners are potentially at greater risk (Safe 1990). During the current study, starling carcasses at all sites, with the exception of the AX reference site during 1999, had measurable concentrations of mono-ortho congeners 105 and 118. As with other PCB congeners measured, concentrations of these co-planar congeners decreased in starling chick carcasses at all sites from 1998 to 1999, and were at least an order of magnitude lower at the AB remediated site in 1999 compared to concentrations measured at that site in 1996.

In evaluation of PCB toxicity, TEQs are often calculated to provide a comparison among study sites. The sum of TEQs were greater at remediated sites compared to reference sites during both 1998 and 1999; which was expected given the greater congener concentrations measured at the remediated sites. However, the lack of measured differences in reproduction and other indicators of adverse biological health among remediated and reference sites suggests that PCB concentrations and TEQ values are below that associated with adverse effects in starlings.

Liver EROD activity did not prove to be a valuable indicator of PCB exposure in starling chicks. Liver EROD activity decreased from pre-remediation (1996) to post-remediation (1998) at all sites. However, post-remediation EROD activity measured during 1999 at both the AL and AB sites, were greater then EROD activity measured at the AB site (155 pmol/mg protein/min) prior to remediation. The AX reference site also had an increase in EROD activity from pre-remediation (1996) to post-remediation (1999). Because PCB concentrations decreased from 1998 to 1999, it does not appear that the increase in EROD activity was related to environmental PCBs. In addition, liver EROD activity, neither before nor after remediation, exhibited the several-fold increase in activity that would be consistent with a useful biomarker response. It does not appear that starling chick exposure to PCBs was sufficient to illicit a response in liver EROD activity, or perhaps EROD activity is not an accurate indicator of PCB contamination in

starlings.

Reproductive Data

There was no difference in adult starling nesting behavior between the remediated and the AX reference site after remediation. Because of the small number of nests established at the AP reference site ($n = 6$, total for both years) data from this site was not used in analysis of reproduction. Because the AP reference site was heavily vegetated and short grassland (pastures, mown fields, lawns, etc) is the starling's preferred habitat, vegetation height may have influenced nest selection at this site (Cabe 1993; Feare 1984).

Remediated sites had hatch rates that ranged between 64 and 79% during 1998 and 1999; hatch rates at the AX reference site were similar, ranging from 67 - 82%. Prior to remediation, starling mean hatch rates also were similar among sites (90 and 81% for reference and PCB sites, respectively) (Arenal 1997). Slightly higher hatch rates (>80%) have been reported in starlings from New York (Kessel 1957).

Fledging success also was similar among study and reference sites during 1998 and 1999, ranging from 44 - 65% at remediated sites and from 50 - 58% at the AX reference site. Prior to remediation, mean fledging success was less at PCB sites compared to reference sites (Arenal 1997). Starling studies at Ithaca, New York, reported greater fledging success (85.2%) than observed in the current study (Kessel 1957).

Behavior

Although post-remediation visitation rates of adult starlings to nest boxes were similar among study and reference sites, visitation rates were generally less after remediation than they were prior to remediation. Prior to remediation, foraging trips among contaminated and reference sites were similar in the first 5 days post-hatch; however, foraging trips during days 6-10 were greater at the reference sites, and decreased at PCB sites from days 6-10 to days 11-15 post-hatch. Our studies conducted prior to remediation indicated a reduction in incubation behavior ($P < 0.5$) from 15.3 counts/hour at reference sites to 10.6 counts/hr at PCB sites during days 11-15 post hatch (Arenal 1997). This suggested decreased parental care at the PCB sites

prior to remediation (Arenal 1997).

McKee (1993) also studied the effects of PCBs on starlings at the AL site prior to remediation and recorded erratic incubation behaviors. He suggested that parental inattentiveness, potentially due to PCB contamination, may have been the principal cause of reduced hatching success.

McCarty and Secord (1999) reported that differences in nest quality among sites were consistent with the hypothesis that PCB contamination had adverse effects on tree swallow behavior. Swallow nests from uncontaminated site were of the highest quality and those from highly contaminated sites were of poorest quality. Differences in quality of nests was not observed in the current study. Only a few starlings exhibited poor nest quality, and these nest were not associated with a particular site. It is not known whether starling nest construction would be affected by PCBs; however, prior to remediation Arenal (1997) reported only 1 of 11 (9%) initiated nests at the AL site (the site with the greatest soil PCB concentrations) proceeded to a full cup (nest completion) compared to 57-69% among the AB site and 2 reference sites.

Body Weights

In the current study, 3-day-old starling chick weights differed ($P < 0.001$) between years, but not among sites. During 1998, 3-day chick weights were similar to 3-day weights (20 g) reported by Arenal (1997) at both remediated and reference sites prior to remediation. Similarly, 3-day chick weights measured in 1999 were similar to 3-day weights (14 g) reported for starlings in North America (Kessel 1957). Three-day weights may have varied between 1998 and 1999 due to fluctuations in environmental conditions (drought in 1999). Also, the nesting season started earlier in 1999 than in 1998, and this may have effected the availability of starling food items.

Similarly, there was a statistical difference in weight of 9-day-old chicks between 1998 (62 g) and 1999 (57 g). However, 9-day-old weights measured after remediation were similar to 9-day weights (62 g) reported by Arenal (1997) prior to remediation. Nine-day weights of starlings pre- and post-remediation were greater than 9-day-old chick weights (50 g) reported by Kessel (1957) for North American starlings.

Although there were statistical differences in 15-day-old male and female chicks weights after remediation, this difference was consistent among sites. Differences in male chick weights between years (Table 5), and among sites for females (Table 6), ranged from 63 to 70 g and were similar to mean 15-day-old starling chick weights reported by Arenal (1997; 65 g) and Kessel (1957; 64 g). Because 3- and 9-day-old chick weights were statistically greater in 1998 compared to 1999 and the reverse was true for 15-day-old males (males in 1999 weighed more than those in 1998), while females did not differ between years, and because 15-day-old chick weights were in general agreement with previously reported weights for similar age starling chicks, the observed differences are not considered to be biologically significant.

Liver weights (mean liver somatic index) of 15-day-old starling chicks differed between reference and remediated sites during 1998 and 1999. However, there was considerable fluctuation in this index among sites between years (Table 7). Kubiak et al. (1989) reported an increased liver to body weight ratio in Forster's terns from a contaminated area in Green Bay, Wisconsin, compared to a reference location at Lake Poygan, Wisconsin. However, there were no indications of enlarged liver weights in tree swallows from sites designated as "Areas of Concern" (areas with persistent contamination, eutrophication, and habitat loss) in the Great Lakes basin (Bishop et al. 1999). An enlarged liver to body weight ratio is often indicative of contaminant exposure (Kubiak et al. 1989), however, the variability in liver weights observed in starling chicks after remediation does not support a conclusion of PCB induced effect.

Hematocrit Data

Mean hematocrit values differed among sites during the current study. There was a trend towards greater hematocrit values in chicks from reference sites, although the difference was not consistent between years. During 1998, hematocrits at the AP reference site were significantly greater than those at the AB remediated site, while during 1999, hematocrit values at AP site were significantly greater than those from the remediated AL site. Prior to remediation, mean hematocrit values at the reference sites (34%) were similar to hematocrit values at PCB sites (36%) (Arenal 1997). Despite the difference in hematocrit values among sites after remediation (approximately 34% at remediated sites and 39% at reference sites), there was no evidence of

anemia at any of the sites. The reason for difference in mean hematocrit values among sites is unknown but does not appear to be associated with adverse effects and may reflect normal fluctuations in this biological parameter.

CONCLUSION

Nesting behavior, productivity, and physical condition of starlings were evaluated between reference and PCB remediated sites. Nest box usage was similar among all sites, with the exception of the AP reference site where habitat conditions were not favorable for nesting. Adult starling nest attentiveness (provisioning rate) was similar among sites. Egg hatch rates and fledging success also were similar among reference and remediated sites. Weight of 3- and 9-day-old chicks did not differ among sites, although they did differ between years. Fifteen-day weights for male starlings chicks also were similar among sites; however, they did differ between years. Contrarily, female 15-day weights did differ among sites, but not between years.

Pre- and post-remedial PCB concentrations and liver EROD activity also were evaluated. There was a reduction in Aroclor 1254 and sum of PCB concentrations in starling chick carcasses between pre- and post-remediation. However, Aroclor 1254 and sum of PCB concentrations in starling chick carcasses at remediated sites were still significantly greater than those measured in starling carcasses at reference sites.

Although liver EROD activity measured in starling chicks prior to remediation was greater than that measured in chicks during 1998 (following remediation), but less than EROD activity measured in chicks during 1999, activity levels tended to be less at reference sites. During 1998 liver EROD activity at both remediated sites was significantly greater than activity measured at the reference site, however during 1999, liver EROD activity only differed between the reference and AL remediated sites.

Our studies indicated that PCB concentrations have decreased in starling chicks at remediated sites relative to concentrations measured at these sites prior to remediation. Even though concentrations at remediated sites remain greater than concentrations measured at reference sites, there has been a concomitant decrease in factors potentially associated with adverse effects. Parental care, egg hatching rates, and fledging success suggest a similarity

between starlings nesting at remediated and reference sites. These results indicate that adverse reproduction and behavioral effects reported by Arenal and Halbrook (1997) may no longer be of concern to avian species utilizing remediated habitats on CONWR. The use of the starling as a model for evaluating potential effects of contaminants on avian species has been successful and demonstrates the value of biological monitoring as a mechanism for evaluating environmental contamination and the efficacy of remediation.

Literature Cited

Ahlborg, U. G., G. C. Becking, L. S. Birnbaum, A. Brouwer, H. J. G. M. Derks, M. Feely, G. Golor, A. Hanberg, J. C. Larsen, A. K. D. Liem, S. H. Safe, C. Schlatter, F. Waern, M. Younes, and E. Yrjankeikke. 1994. Toxic equivalency factors for dioxin-like PCBs. Chemosphere 28:1049-1067.

Arenal, C. A. 1997. European starling (Sturnus vulgaris):Avian model and monitor of contaminant and remedial effects at Crab Orchard National Wildlife Refuge. Thesis, Southern Illinois University, Carbondale, Illinois, USA.

Arenal, C. A. and R. S. Halbrook. 1997. PCB and heavy metal contamination and effects in European starlings (Sturnus vulgaris) at a Superfund site. Bulletin of Environmental Contamination and Toxicology 58:254-262.

Bishop, C. A., N. A. Mahony, S. Trudeau, and K. E. Pettit. 1999. Reproductive success and biochemical effects in three swallows (Tachycineta bicolor) exposed to chlorinated hydrocarbon contaminants in wetlands of the Great Lakes and St. Lawrence River Basin, USA and Canada. Environmental Toxicology and Chemistry 18:263-271.

Cabe, P. R. 1993. European starling (Sturnus vulgaris). Pages 1-24 in A. Poole and F. Gill, eds. The birds of North America, No. 48. The Academy of Natural Sciences, Washington, D.C.: The American Ornithologists' Union.

Falandysz, J., N. Yamashita, S. Tanabe, R. Tatsukawa, L. Rucinska, T. Mizera, and B. Jakuczun. 1994. Congener-specific analysis of polychlorinated biphenyls in white-tailed sea eagles Haliaeetus albicilla collected in Poland. Archives of Environmental Contamination and Toxicology 26:3-22.

Feare, C. 1984. The Starling. Oxford University Press, New York, New York, USA.

Fox, G. A. 1993. What have biomarkers told us about the effects of contaminants on the health of fish-eating birds in the Great Lakes? The theory and a literature review. International Association of Great Lakes Research 19:722-736.

Furness, R. W. 1993. Birds as monitors of pollutants. Pages 860143 in R. W Furness and J. J. D. Greenwook, eds. Birds as monitors of environmental change. Chapman and Hall, London.

Grant, D. L., W. E. J. Phillips, and D. C. Villeneuve. 1971. Metabolism of a polychlorinated biphenyl (Aroclor 1254) mixture in the rat. Bulletin of Environmental Contamination and Toxicology 6:102-112.

Kessel, B. 1957. A study of the breeding biology of the European starling in North America. American Midland Naturalist 58:257-331.

Kubiak, T. J., H. J. Harris, L. M. Smith, T. R. Schwartz, D. L. Stalling, J. A. Trick, L. Sileo, D. E. Docherty, and T. C. Erdman. 1989. Microcontaminants and reproductive impairment of the Forster's tern on Green Bay, Lake Michigan-1983. Archives of Environmental Contamination and Toxicology 18:706-727.

Lumbardo, G. 1993. PCB Areas Operable Unit Crab Orchard National Wildlife Refuge. Fact Sheet Number 2, Schlumberger Industries, Inc., c/o Crab Orchard National Wildlife Refuge, Marion, Illinois. 6 pp.

Lubet, R. A., R. W. Nims, R. T. Mayer, J. W. Cameron, and I. M. Schechtman. 1985. Measurement of cytochrome P-450 dependent dealkylation of alkoxyphenoxasones in hepatic S9s and hepatocyte homogenates: effects of dicumarol. Mutation Research 142:127-131.

Mazel, P. 1971. Experiments illustrating drug metabolism in vitro. Pages 546-560 in B. N. La Du, H. G. Mandel, and E. L. Ways, eds. Fundamentals of drug metabolism and drug disposition. Williams and Wilkens, Baltimore, Maryland, USA.

McCarty, J. P. and A. L. Secord. 1999. Nest-building behavior in PCB-contaminated tree swallows. The Auk 116:55-63.

McKee, M. J. 1993, Biological monitoring of chemical contamination at Crab Orchard National Wildlife Refuge. Draft Final Report, Submitted to Refuge Manager, Crab Orchard National Wildlife Refuge, Carterville, Illinois, USA.

Mora, M. A. 1996. Congener-specific polychlorinated biphenyl patterns in eggs of aquatic birds from the lower Laguna Madre, Texas. Environmental Toxicology and Chemistry 15:1003-1010.

O'Brien and Gere Engineers, Inc. 1988. Remedial investigation report, volume 1: Report. O'Brien and Gere Engineers, Inc., Syracuse, New York.

Safe, S. H. 1990. Polychlorinated biphenyls (PCBs): Biochemistry, toxicology, and mechanism of action. CRC Critical Reviews in Toxicology 13:319-395.

Selikoff, I. J. 1972. PCBs-Environmental Impact. Environmental Research 5:249-362.

Stickel, W. H., L. F. Stickel, R. A. Dyrland, and D. L. Hughes. 1984. Aroclor 1254 residues in birds: lethal levels and loss rates. Archives of Environmental Contamination and Toxicology 13:7-12.

U.S. Environmental Protection Agency. 1988. Remedial Investigation Summary: Crab Orchard National Wildlife Refuge Williamson County, Illinois. Office of Public Affairs, Region 5, Chicago, Illinois.

_____. 1990. Declaration for the record of decision: Crab Orchard National Wildlife Refuge PCB Areas Operable Unit. Office of Public Affairs, Region 5, Chicago, Illinois.

_____. 1992. Test methods for evaluating solid waste, physical/chemical methods, SW-846, Third edition. Organic Section. Automated soxhlet extraction (Method 3541), gel permeation clean-up (Method 3640), and organochlorine pesticides and PCBs (Method 8081). U.S. Environmental Protection Agency, Washington D.C., USA.

Table 1. Total number of starling nests that produced at least one egg/number of nest boxes at the Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference (AP) sites on Crab Orchard National Wildlife Refuge and at the Annex reference (AX) site on the campus of Southern Illinois University during 1998 and 1999.

	Site			
Year	AB	AL	AP	AX
1998	15/12	18/12	6/20	36/24
1999	17/12	17/12	1/20	27/24

Table 2. Mean clutch size, percent hatching success, and percent chicks surviving 15-days post-hatch (± SE) at the Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference (AP) sites on Crab Orchard National Wildlife Refuge and at the Annex reference (AX) site on the campus of Southern Illinois University during 1998 and 1999.

	Clutch size		Hatching success (%)		Survival rate (%)	
Site	1998	1999	1998	1999	1998	1999
AB	4.93 ± 0.13	4.63 ± 0.18	70 ± 10	64 ± 9	65 ± 13	44 ± 11
AL	5.00 ± 0.17	5.12 ± 0.23	64 ± 8	79 ± 6	50 ± 12	59 ± 11
AP	5.00 ± 0.00	5.00[a]	93 ± 7	100	91 ± 6	100
AX	4.97 ± 0.17	4.96 ± 0.16	67 ± 7	82 ± 6	50 ± 9	58 ± 9

[a]Only one clutch was laid.

Table 3. Mean visitation rate[a] (± SE) for adult starlings at the Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference (AP) sites at Crab Orchard National Wildlife Refuge and at the Annex reference (AX) site on the campus of Southern Illinois University during 1998 and 1999.

Site	Mean visitation rate
AB ($n = 130$)[b]	3.8 ± 0.28
AL ($n = 106$)	3.0 ± 0.30
AP ($n = 30$)	5.5 ± 0.70
AX ($n = 177$)	3.7 ± 0.25

[a]Visitation rate is defined as the number of times adults come to the nest per half hour after chicks hatched.
[b]Number of half hour observation periods at each site.

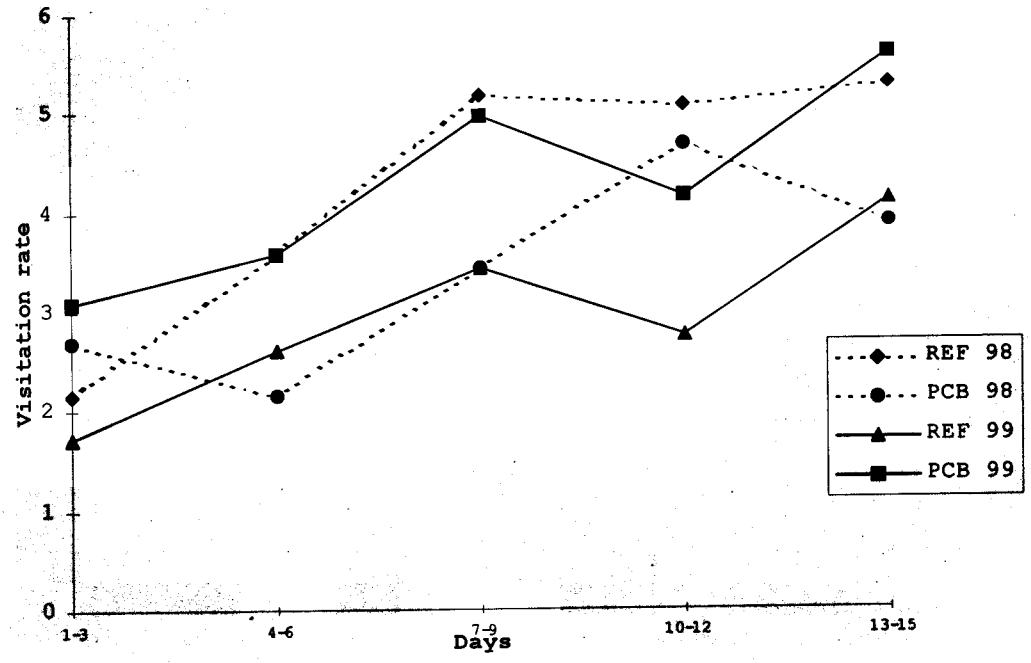

Figure 1. Mean visitation rates (number of times adults came to the nest per half hour) of starlings during various time periods at remediated (PCB, Crab Orchard National Wildlife Refuge Area 9 Building Complex and Area 9 Landfill) and reference (REF, Crab Orchard National Wildlife Refuge Area P and AX reference located on the campus of Southern Illinois University) sites during 1998 and 1999.

Table 4. Mean weight (g ± SE) of 3- and 9-day-old starling chicks from the Area 9 Building Complex, Area 9 Landfill, and Area P reference sites on Crab Orchard National Wildlife Refuge and Annex reference site on the campus of Southern Illinois University during 1998 and 1999.

Year	3-day	9-day
1998	22.38 ± 0.55	62.34 ± 0.75
1999	16.24 ± 0.42	57.27 ± 0.84
P-value[a]	< 0.001	0.008

[a]Two-way ANOVA between years.

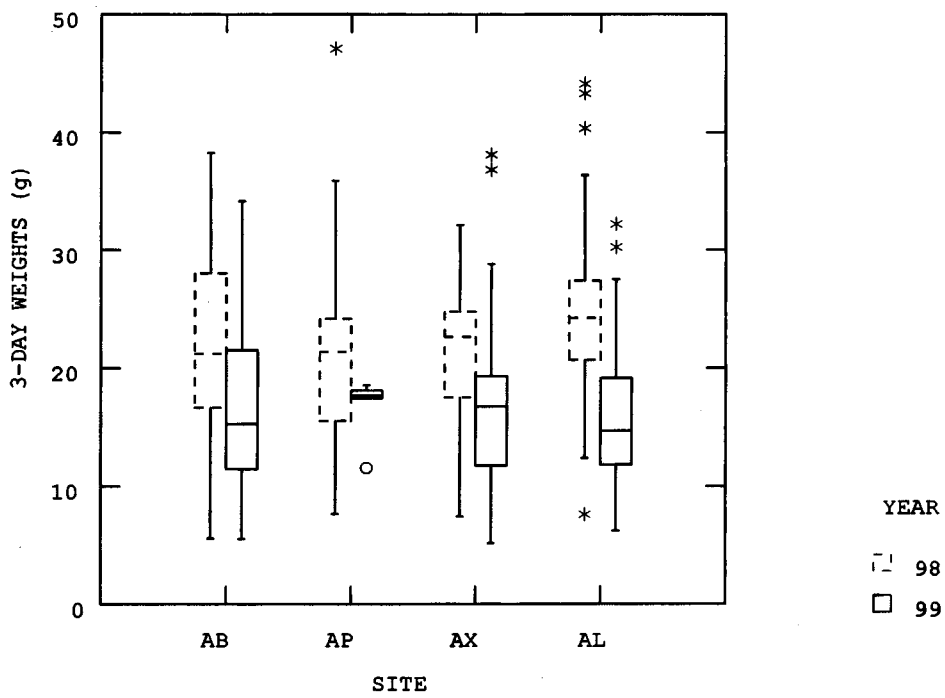

Figure 2. Box Plots of weights of 3-day-old starling chicks collected from the Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference sites (AP) on Crab Orchard National Wildlife Refuge and the Annex (AX) reference site on the campus of Southern Illinois University during 1998 and 1999. (* = outside outlier, o = far outside outlier)

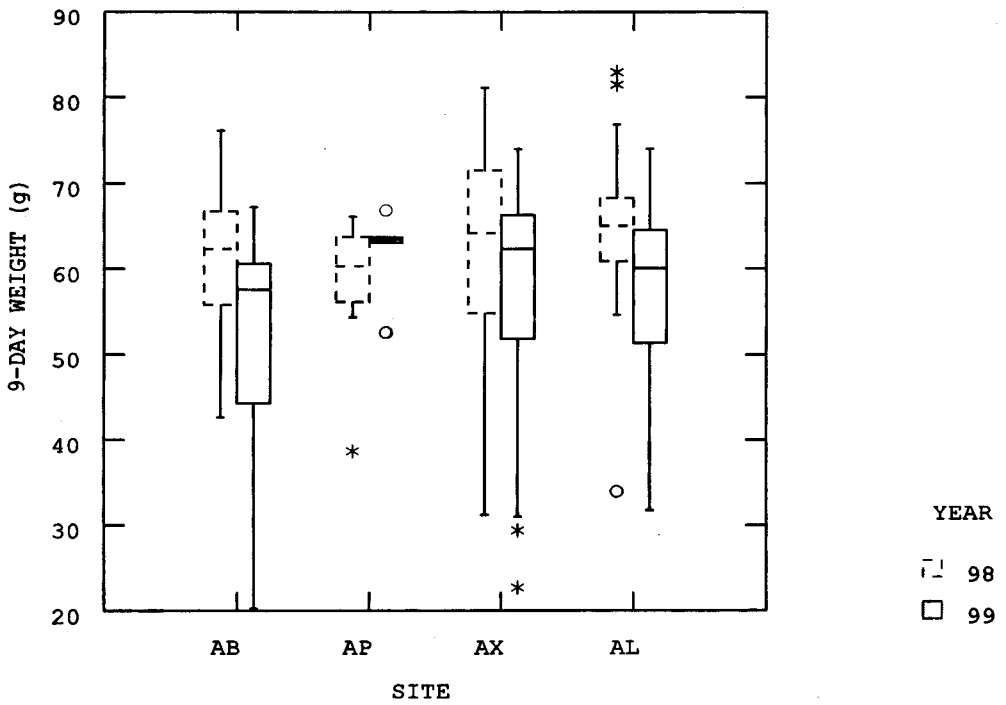

Figure 3. Box Plots of weights of 9-day-old starling chicks from the Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference sites (AP) on Crab Orchard National Wildlife Refuge and the Annex (AX) reference site on the campus of Southern Illinois University during 1998 and 1999. (* = outside outlier, o = far outside outlier)

Table 5. Mean body weights (g ± SE) of male 15-day-old starling chick carcasses collected from Area 9 Building Complex, Area 9 Landfill, and Area P reference sites on Crab Orchard National Wildlife Refuge and from the Annex reference site on the campus of Southern Illinois University during 1998 and 1999.

Year	Male body weights
1998	67.37 ± 0.82
1999	70.12 ± 0.87
P-value[a]	0.015

[a]Two-way ANOVA between years.

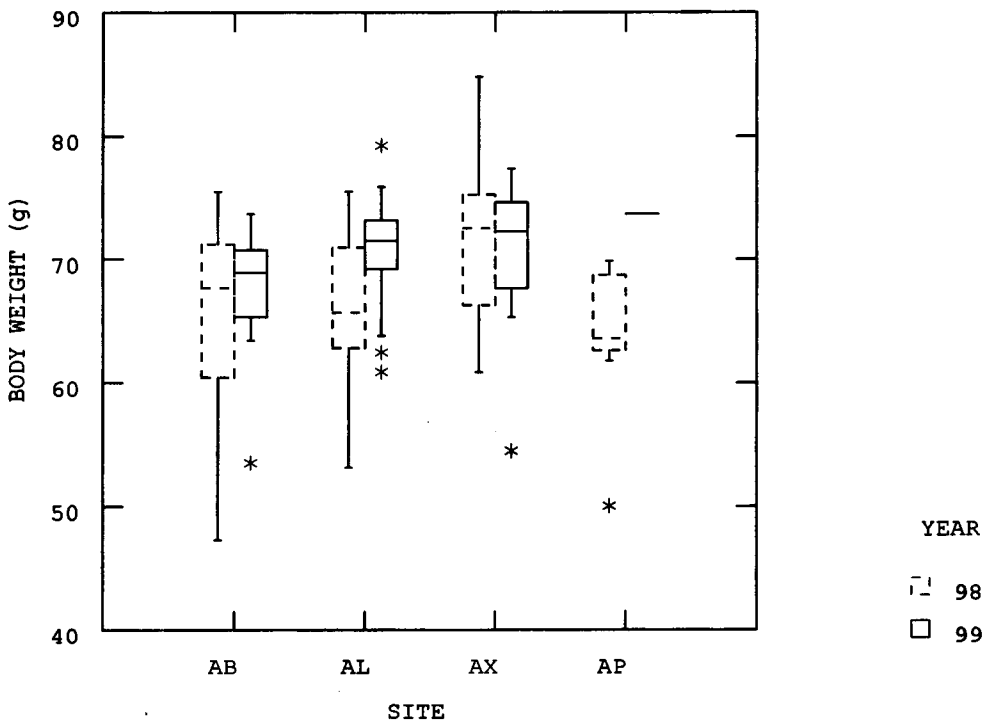

Figure 4. Box Plots of weights of male 15-day-old starling chicks collected from the Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference sites (AP) on Crab Orchard National Wildlife Refuge and the Annex (AX) reference site on the campus of Southern Illinois University during 1998 and 1999. (* = outside outlier)

Table 6. Mean body weights (g (± SE) of female 15-day-old starling chick carcasses collected from Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference (AP) sites on Crab Orchard National Wildlife Refuge and from the Annex reference site (AX) on the campus of Southern Illinois University during 1998 and 1999.

Site	Female body weights
AB	62.91 ± 1.13 A[a]
AL	66.84 ± 0.89 B
AP	65.62 ± 1.38 AB
AX	69.35 ± 0.78 B
P-value[b]	0.002

[a]Mean body weights sharing the same letter are not significantly different.
[b]Two-way ANOVA among sites.

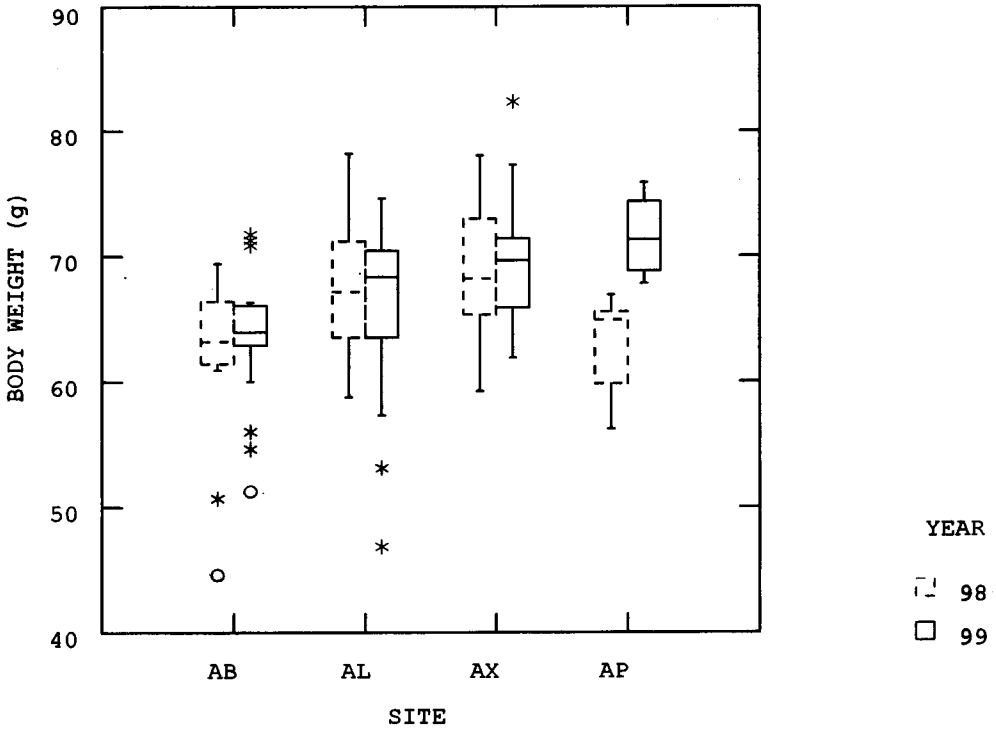

Figure 5. Box Plots of weights of female 15-day-old starling chicks collected from the Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference sites (AP) on Crab Orchard National Wildlife Refuge and the Annex (AX) reference site on the campus of Southern Illinois University during 1998 and 1999. (* = outside outlier, o = far outside outlier)

Table 7. Mean liver somatic index[a] (± SE) of 15-day-old starling chick carcasses collected from Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference (AP) sites on Crab Orchard National Wildlife Refuge and from the Annex reference site (AX) on the campus of Southern Illinois University during 1998 and 1999.

Site	1998	1999	*P*-value[b]
AB	6.27 ± 0.15 A[c] (*n* = 32)[d] A[e]	5.67 ± 0.19 AB (*n* = 25) A	0.100
AL	5.51 ± 0.10 B (*n* = 36) A	5.54 ± 0.10 A (*n* = 47) A	1.000
AP	5.68 ± 0.26 AB (*n* = 19) A	5.04 ± 0.18 AB (*n* = 5) A	0.769
AX	5.45 ± 0.10 B (*n* = 56) A	6.05 ± 0.18 B (*n* = 33) B	0.021
P-value[f]	<0.001	0.021	

[a]Somatic index = [(liver weight/body weight) * 100]
[b]Tukey-Kramer multiple comparison analysis between years.
[c]Different capital letters to the right of means indicate differences between sites within years (i.e. within columns).
[d]Number of starling chick livers analyzed from each site.
[e]Different capital letters below means indicate differences between years within sites (i.e. across rows).
[f]ANOVA among sites.

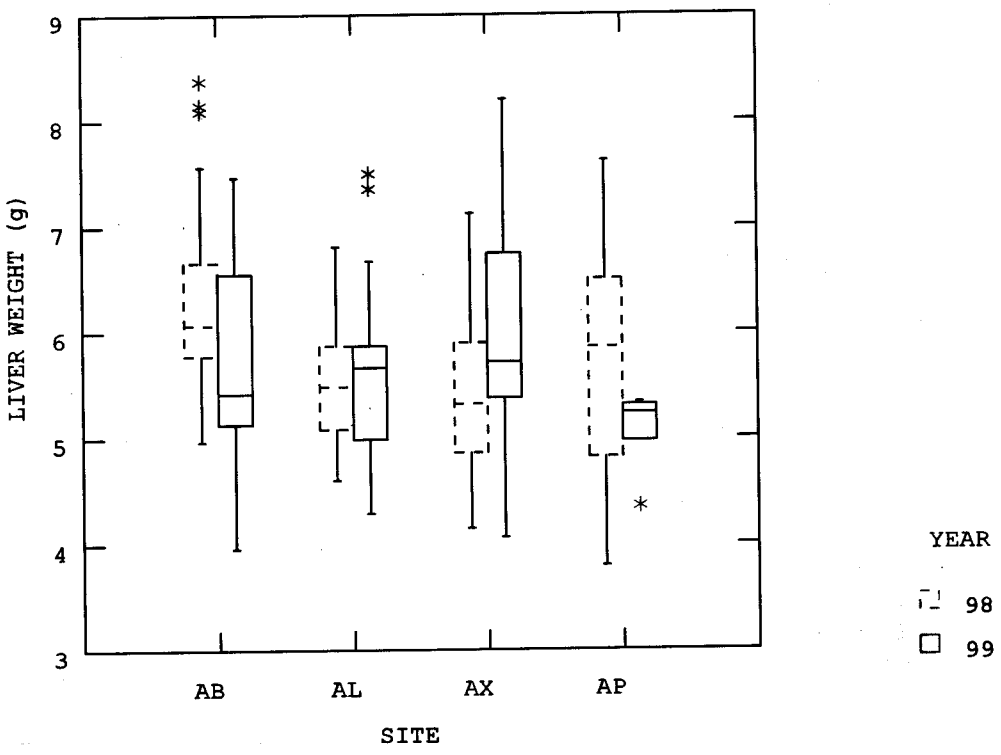

Figure 6. Box Plots of liver somatic indexes of 15-day-old starling chicks collected from the Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference sites (AP) on Crab Orchard National Wildlife Refuge and the Annex (AX) reference site on the campus of Southern Illinois University during 1998 and 1999. (* = outside outlier)

Table 8. Mean PCB congener concentrations (mg/kg) and toxic equivalent quotients (TEQs[a]) of 15-day-old starling chick carcasses collected from the Area 9 Landfill (AL) and Area 9 Building Complex (AB) on Crab Orchard National Wildlife Refuge during 1998.

		AB (n = 38)		AL (n = 37)		
Congener	TEF value[b]	mg/kg	TEQ	mg/kg	TEQ	P-value[c]
18	0.00002	NQ[d]	-	NQ	-	NA[e]
31	0.000001	NQ	-	NQ	-	NA
52	0.00002	0.025	$5.0E^{-7}$	0.030	$6.6E^{-7}$	0.562
49	0.00002	NQ	-	NQ	-	NA
44	0.00002	NQ	-	NQ	-	NA
101	0.00002	0.038	$7.6E^{-7}$	0.049	$9.8E^{-7}$	0.333
99	0.00002	0.031	$6.2E^{-7}$	0.042	$8.4E^{-7}$	0.169
87	0.00002	NQ	-	0.138	$2.8E^{-6}$	NA
110	0.00002	NQ	-	NQ	-	NA
151	0.00002	NQ	-	NQ	-	NA
118	0.001	0.073	$7.3E^{-5}$	0.092	$9.2E^{-5}$	0.392
105	0.001	0.020	$2.0E^{-5}$	0.025	$2.5E^{-5}$	0.565
128	0.00002	0.009	$1.8E^{-7}$	0.022	$4.4E^{-7}$	0.063
126	0.1	NQ	-	NQ	-	NA
156	0.00002	NQ	-	NQ	-	NA
169	0.05	NQ	-	NQ	-	NA
170	0.00002	NQ	-	NQ	-	NA
153	0.00002	0.081	$1.6E^{-6}$	0.110	$2.2E^{-6}$	0.082
138	0.00002	0.068	$1.4E^{-6}$	0.093	$1.9E^{-6}$	0.144
180	0.00002	NQ	-	NQ	-	NA
Sum[f]		2.372	$1.0E^{-4}$	3.113	$1.3E^{-4}$	0.301

[a]Toxic equivalent quotients calculated as TEF × congener concentration (mg/kg)
[b]Toxic equivalent factors as reported by Safe (1990) and Ahlborg et al. (1994)
[c]Student's t-test for PCB congener concentrations between sites (calculated only when >50% of the samples had concentrations above the detection limit; when calculated, a value of ½ detection limit was used for samples with concentration below the detection limit)
[d]<50% of samples had detectable concentrations of the individual congener
[e]Not applicable
[f]Sum of all congeners in sample (including those not listed)

Table 9. Mean PCB congener concentrations (mg/kg) and toxic equivalent quotients (TEQs[a]) of 15-day-old starling chick carcasses collected from the Area 9 Landfill (AL) and Area 9 Building Complex (AB) on Crab Orchard National Wildlife Refuge during 1999.

		AB (n = 25)		AL (n = 26)		
Congener	TEF value[b]	mg/kg	TEQ	mg/kg	TEQ	P-value[c]
18	0.00002	NQ[d]	-	NQ	-	NA[e]
31	0.000001	NQ	-	NQ	-	NA
52	0.00002	NQ	-	NQ	-	NA
49	0.00002	NQ	-	NQ	-	NA
44	0.00002	NQ	-	NQ	-	NA
101	0.00002	0.033	$6.6E^{-7}$	0.034	$6.8E^{-7}$	0.947
99	0.00002	0.027	$5.4E^{-7}$	0.021	$4.2E^{-7}$	0.114
87	0.00002	NQ	-	NQ	-	NA
110	0.00002	NQ	-	NQ	-	NA
151	0.00002	NQ	-	NQ	-	NA
118	0.001	0.035	$3.5E^{-5}$	0.040	$4.0E^{-5}$	0.443
105	0.001	0.009	$9.0E^{-6}$	0.010	$1.0E^{-5}$	0.373
128	0.00002	0.033	$6.6E^{-7}$	0.028	$5.6E^{-7}$	0.458
126	0.1	NQ	-	NQ	-	NA
156	0.00002	NQ	-	NQ	-	NA
169	0.05	NQ	-	NQ	-	NA
170	0.00002	NQ	-	NQ	-	NA
153	0.00002	0.041	$8.2E^{-7}$	0.034	$6.8E^{-7}$	0.276
138	0.00002	0.022	$4.4E^{-7}$	0.035	$7.0E^{-7}$	0.103
180	0.00002	NQ	-	NQ	-	NA
Sum[f]		1.188	$4.7E^{-5}$	1.179	$5.3E^{-5}$	0.968

[a]Toxic equivalent quotients calculated as TEF × congener concentration (mg/kg)
[b]Toxic equivalent factors as reported by Safe (1990) and Ahlborg et al. (1994)
[c]Student's t-test for PCB congener concentrations between sites (calculated only when >50% of the samples had concentrations above the detection limit; when calculated, a value of ½ detection limit was used for samples with concentration below the detection limit)
[d]<50% of samples had detectable levels of the individual congener
[e]Not applicable
[f]Sum of all congeners in sample (including those not listed)

Table 10. Mean PCB congener concentrations (mg/kg) and toxic equivalent quotients (TEQs[a]) of 15-day-old starling chicks collected from the Area P reference (AP) site on Crab Orchard National Wildlife Refuge during 1998 and from the Annex reference (AX) site on the campus of Southern Illinois University during 1999.

		1998 AP (n = 19)		1999 AX (n = 24)		
Congener	TEF value[b]	mg/kg	TEQ	mg/kg	TEQ	P-value[c]
18	0.00002	NQ[d]	-	NQ	-	NA[e]
31	0.000001	NQ	-	NQ	-	NA
52	0.00002	0.019	$3.8E^{-7}$	NQ	-	NA
49	0.00002	NQ	-	0.006	$1.2E^{-7}$	NA
44	0.00002	NQ	-	NQ	-	NA
101	0.00002	NQ	-	NQ	-	NA
99	0.00002	0.019	$3.8E^{-7}$	NQ	-	NA
87	0.00002	NQ	-	NQ	-	NA
110	0.00002	NQ	-	NQ	-	NA
151	0.00002	NQ	-	NQ	-	NA
118	0.001	0.016	$1.6E^{-5}$	NQ	-	NA
105	0.001	0.013	$1.3E^{-5}$	NQ	-	NA
128	0.00002	NQ	-	NQ	-	NA
126	0.1	NQ	-	NQ	-	NA
156	0.00002	NQ	-	NQ	-	NA
169	0.05	NQ	-	NQ	-	NA
170	0.00002	NQ	-	NQ	-	NA
153	0.00002	NQ	-	NQ	-	NA
138	0.00002	NQ	-	NQ	-	NA
180	0.00002	NQ	-	NQ	-	NA
Sum[f]		0.448	$3.0E^{-5}$	0.199	$1.2E^{-7}$	0.439

[a]Toxic equivalent quotients calculated as TEF × congener concentration (mg/kg)
[b]Toxic equivalent factors as reported by Safe (1990) and Ahlborg et al. (1994)
[c]Student's T-test for PCB congener concentrations between sites (calculated only when >50% of the samples had concentrations above the detection limit; when calculated, a value of ½ detection limit was used for samples with concentration below the detection limit)
[d]<50% of samples had detectable levels of the individual congener
[e]Not applicable
[f]Sum of all congeners in sample (including those not listed)

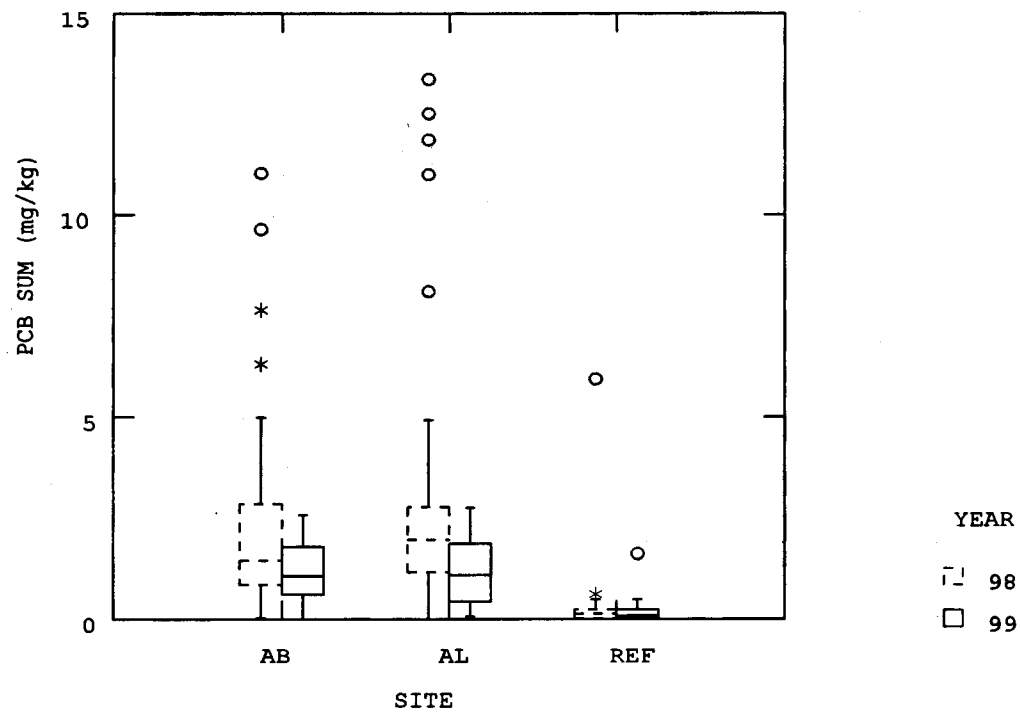

Figure 7. Box Plots of sum of PCB congener concentrations in carcasses of 15-day-old starling chicks from the remediated Area 9 Building Complex (AB) and Area 9 Landfill (AL) on Crab Orchard National Wildlife Refuge, and reference sites (REF; Area P reference site on Crab Orchard National Wildlife Refuge during 1998 and Annex reference site on the campus of Southern Illinois University during 1999) during 1998 and 1999. (* = outside outlier, o = far outside outlier)

Table 11. Aroclor 1254 concentrations (mg/kg ± SE) in 15-day-old starling chick carcasses collected from Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference (AP) sites on Crab Orchard National Wildlife Refuge and from the Annex reference site (AX) on the campus of Southern Illinois University during 1998 and 1999.

Site	Aroclor 1254
AB	2.24 ± 0.19 A[a]
AL	2.71 ± 0.31 A
AP	0.213 ± 0.05 B
AX	0.482 ± 0.09 B
P-value[b]	<0.001

[a]Mean Aroclor 1254 concentrations sharing the same letter are not significantly different.
[b]Two-way ANOVA among sites.

Table 12. Mean (SE) total PCB concentrations (mg/kg) measured in starling chicks that died prior to 15-days of age and collected from remediated site on Crab Orchard National Wildlife Refuge [Area 9 Building Complex (AB) and Area 9 Landfill (AL)] and from a reference site located on the campus of Southern Illinois University, Carbondale, IL (AX) during 1998 and 1999.

Site	1998	1999
AX	0.29 (0.17) $n = 7$	0.10 (0.02) $n = 2$
AB	1.62 (0.98) $n = 3$	2.13 (1.18) $n = 10$
AL	4.33 (1.46) $n = 10$	1.88 (0.78) $n = 4$
Pvalue[a]	<0.001	0.082

[a]Kruskal-Wallis

Table 13. Mean (SE) total PCB concentrations (mg/kg) measured in eggs that did not hatch and collected from remediated site on Crab Orchard National Wildlife Refuge [Area 9 Building Complex (AB) and Area 9 Landfill (AL)] and from a reference site located on the campus of Southern Illinois University, Carbondale, IL (AX) during 1998 and 1999.

Site	1998	1999
AX	1.05 (0.54) $n = 5$	0.61 (0.74) $n = 5$
AB	11.88 (5.74) $n = 5$	6.30 (4.12) $n = 7$
AL	5.14 (5.03) $n = 7$	5.01 (3.70) $n = 6$
Pvalue[a]	0.015	0.006

[a]Kruskal-Wallis

Table 14. Mean ethoxyresorufin O-deethylase (EROD) activity (pmole/mg protein/min ± SE) in liver of 15-day-old starling chick carcasses collected from the Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference (AP) sites on Crab Orchard National Wildlife Refuge and the Annex reference (AX) site on the campus of Southern Illinois University during 1998 and 1999.

Site	1998	1999	*P*-Value[a]
AB	136 ± 15 A[b] ($n = 33$)[c]	256 ± 36 AB ($n = 12$)	0.001
AL	114 ± 9 A ($n = 36$)	361 ± 18 B ($n = 12$)	<0.001
AX	-[d]	232 ± 49 A ($n = 12$)	<0.001[e]
AP	58 ± 6 B ($n = 14$)	-[d]	
P-value[f]	0.002	0.041	

[a]Tukey multiple comparison analysis between years.
[b]EROD activity values sharing the same letter are not significantly different among sites within years.
[c]Number of chicks that were analyzed for liver EROD activity at each site.
[d]Liver EROD activity was not quantified
[e]Comparison of EROD activity between AP reference site in 1998 and AX reference site in 1999
[f]ANOVA among sites

Table 15. Mean hematocrit values (%) collected from the brachial vein of 15-day-old starling chicks from the Area 9 Building Complex (AB), Area 9 Landfill (AL), and Area P reference (AP) sites on Crab Orchard National Wildlife Refuge and the Annex reference (AX) site on the campus of Southern Illinois University during 1998 and 1999.

Site	1998	1999	*P*-value[a]
AB	32 ± 0.9 A[b] (*n* = 33)[c] A[d]	35 ± 0.9 AB (*n* = 15) A	0.132
AL	36 ± 0.7 B (*n* = 36) A	34 ± 0.6 A (*n* = 41) A	0.177
AX	39 ± 0.7 B (*n* = 55) A	35 ± 0.6 AB (*n* = 28) B	0.003
AP	40 ± 0.6 B (*n* = 17) A	41 ± 0.6 B (*n* = 5) A	1.000
P-value[e]	<0.001	0.001	

[a]Tukey multiple comparison analysis between years.
[b]Different capital letters to the right of means indicate differences between sites within years (i.e. within columns).
[c]Number of chicks that blood was collected from at each site.
[d]Different capital letters below means indicate differences between years within sites (i.e. across rows).
[e]ANOVA among sites.

Table 16. Total PCB and Aroclor 1254 concentrations (mg/kg) in 15-day-old starling chick carcasses analyzed by a United States Fish and Wildlife Service approved Analytical Control Facility (ACF) and the Cooperative Wildlife Research Laboratory (CWRL) on the campus of Southern Illinois University during 1998.

	Total PCBs		Aroclor 1254	
Sample Number	CWRL	ACF	CWRL	ACF
98BC54-2A	2.50	1.41	2.85	1.71
98BC61-1A	1.35	0.98	1.61	1.29
98BC62-2B	0.82	0.20	0.78	0.25
98AP8-4A	0.51	0.41	0.81	0.30
98LF63-1A	11.86	4.02	12.06	5.05
98LF65-2A	1.01	0.83	0.65	1.03
98LF69-3A	2.23	0.99	2.42	1.44
98LF70-2A	2.03	1.46	2.21	1.56
Correlation[a]		0.974		0.977

[a]Pearson correlation coefficient

Table 17. Mean PCB congener concentrations (mg/kg) and toxic equivalent quotients (TEQs[a]) for 15-day-old starling chick carcasses collected from the Area 9 Building Complex (AB) on Crab Orchard National Wildlife Refuge during 1999 (post-remediation) and 1996 (pre-remediation).

		AB 1999 (N=25)		AB 1996 (N=55)		
Congener	TEF value[b]	mg/kg	TEQ	mg/kg	TEQ	*P*-value[c]
18	0.00002	NQ[d]	-	NQ	-	NA[e]
31	0.000001	NQ	-	NQ	-	NA
52	0.00002	NQ	-	0.16	$3.2E^{-6}$	NA
49	0.00002	NQ	-	0.07	$1.4E^{-6}$	NA
44	0.00002	NQ	-	NQ	-	NA
101	0.00002	0.033	$7.0E^{-7}$	0.54	$1.1E^{-5}$	0.001
99	0.00002	0.027	$6.6E^{-7}$	0.42	$8.4E^{-6}$	0.001
87	0.00002	NQ	-	0.09	$1.8E^{-6}$	NA
110	0.00002	NQ	-	0.16	$3.2E^{-6}$	NA
151	0.00002	NQ	-	NQ	-	NA
118	0.001	0.035	$4.3E^{-5}$	0.56	$5.6E^{-4}$	0.001
105	0.001	0.009	$1.0E^{-5}$	0.18	$1.8E^{-4}$	0.001
128	0.00002	0.033	$9.2E^{-7}$	0.15	$3.0E^{-6}$	0.001
126	0.1	NQ	-	NQ	-	NA
156	0.00002	NQ	-	NQ	-	NA
169	0.05	NQ	-	NQ	-	NA
170	0.00002	NQ	-	0.06	$1.2E^{-6}$	NA
153	0.00002	0.041	$8.4E^{-7}$	0.67	$1.3E^{-5}$	0.001
138	0.00002	0.022	$5.2E^{-7}$	0.75	$1.5E^{-5}$	0.001
180	0.00002	NQ	-	0.14	$2.8E^{-6}$	NA
Sum[f]		1.188	$5.7E^{-5}$	4.174	$8.0E^{-4}$	0.001

[a]Toxic equivalent quotients calculated as TEF × congener concentration.
[b]Toxic equivalent factors as reported by Safe (1990) and Ahlborg et al. (1994).
[c]Student's t-test for PCB congener concentrations between 1999 (post-remediation) and 1996 (pre-remediation)(value of ½ detection limit used for concentration below the detection limit).
[d]<50% of samples had detectable levels of the individual congener.
[e]Not applicable.
[f]Sum of all congeners in sample (including those not listed).